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Review

A review of cyanobacterial odorous and bioactive metabolites: Impacts and management alternatives in aquaculture

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ABSTRACT

Increased demand has pushed extensive aquaculture towards intensively operated production systems, commonly resulting in eutrophic conditions and cyanobacterial blooms. This review summarizes those cyanobacterial secondary metabolites that can cause undesirable tastes and odors (odorous metabolites) or are biochemically active (bioactive metabolites) in marine and freshwater, extensive and intensive aquaculture systems. For the scope of this paper, biochemically active metabolites include (1) toxins that can cause mortality in aquaculture organisms or have the potential to harm consumers via accumulation in the product (hepatotoxins, cytotoxins, neurotoxins, dermatoxins, and brine shrimp/molluskal toxins), (2) metabolites that may degrade the nutritional status of aquaculture species (inhibitors of proteases and grazer deterrents) or (3) metabolites that have the potential to negatively affect the general health of aquaculture species or aquaculture laborers (dermatoxins, irritant toxins, hepatotoxins, cytotoxins). Suggestions are made as to future management practices in intensive and extensive aquaculture and the potential exposure pathways to aquaculture species and human consumers are identified.

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1. Cyanobacteria in aquaculture

Aquaculture is the fastest growing animal food sector (FAO, 2007), and cultured fish supply now provides over 13% of the animal protein intake for the human population (WHO, 2007). As catch rates of wild fisheries have leveled off or declined since the 1970s, aquaculture farming has grown to offset the increased demand. This increased demand has pushed aquaculture from extensive aquaculture, in which growth of cultured species is supported by the natural productivity of the water body (at times stimulated with fertilizer additions), towards intensively operated production systems such as high-density ponds and pens (Avault, 1996).

Intensive aquaculture requires high stocking densities and supplemental feed to achieve high productivity per unit water volume, commonly resulting in eutrophic to hypereutrophic conditions (Tucker, 1996; Zimba and Grimm, 2003). For example, during peak feeding periods in Mississippi, USA over 150 kg ha^{-1} of 32% protein fish feed is broadcasted in ponds, leading to over 1 mg of dissolved nitrogen (N) L^{-1} d^{-1} from unassimilated feed and fish excreta. In order to prevent inhibitory/toxic levels of N from being reached in intensive

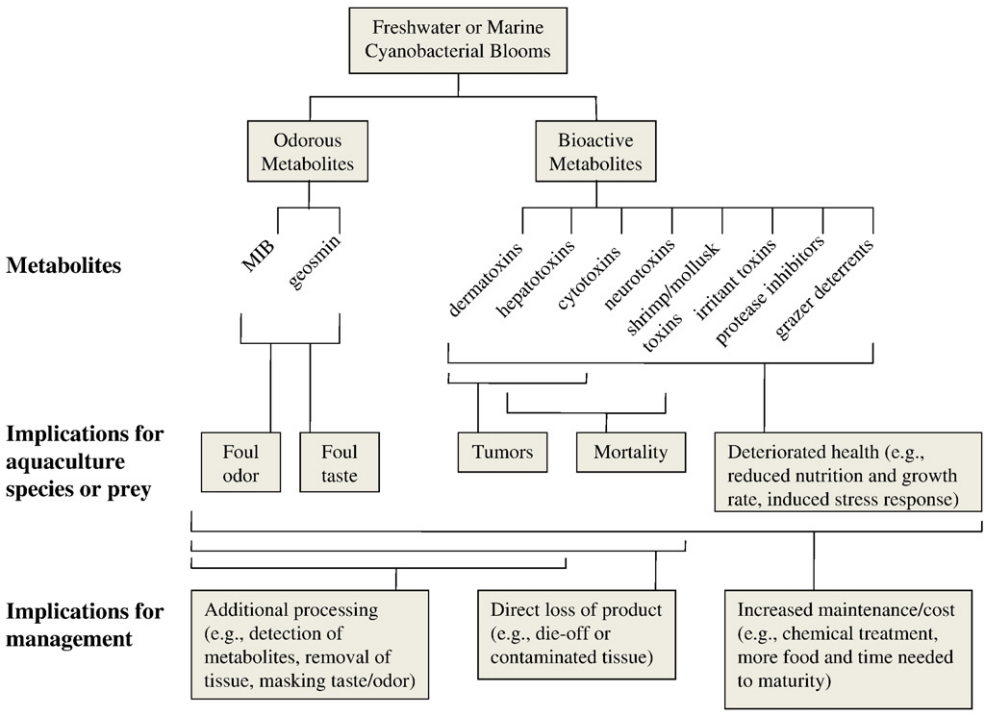

Fig. 1. Possible implications of cyanobacterial secondary metabolites for aquaculture species (cultured crustaceans, mollusks and fish) and management.

systems, microbial populations are necessary for the removal of waste (Avault, 1996). Nitrogen, an essential nutrient for autotrophs, is readily assimilated by phytoplankton as either nitrate or ammonia. The high nutrient levels in these systems result in dense algal blooms that average 75 μg chlorophyll *a* L^{-1} annually and often exceed 300–1000 μg chl *a* L^{-1} (Magalhães et al., 2001; Zimba and Gitelson, 2006). Average phytoplankton cell density can exceed 1×10^6 cells ml^{-1} (Zimba et al., 2001a), with bacteria densities typically over 2×10^6 cells ml^{-1} (Zimba and Mischke, 2005). In both intensive and extensive aquaculture, phytoplankton succession mimics natural systems, with cyanobacterial abundance reaching its maximum in summer; however, the period of high abundance can be prolonged in intensive systems when conditions suitable for growth persist (Raymont, 1980; Payne, 1986; Cichra et al., 1995).

Cyanobacteria (i.e., blue-green algae or cyanoprokaryota) possess several competitive advantages (e.g., resistance to grazing, enhanced nutrient sequestration and light utilization) over eukaryotic taxa which allow the group to dominate under systems of high herbivory and extreme nutrient and light conditions (Dittmann and Wiegand, 2006). Cyanobacteria can be recognized by selective filter-feeding invertebrates as nutritionally poor or toxic and the formation of colonies or elongated filaments can mechanically interfere with grazing. As a result of both processes, grazing is reduced on the less preferred cyanobacterial species and their abundance increases

Table 1
Maximum accumulation of cyanobacterial toxins (NOD=nodularin, MC=microcystins) in aquatic animals in natural systems

Organism	Tissue	Maximum tissue load	Location	Reference
Mollusk				
Bellamya aeruginosa Freshwater snail	Hepatopancreas	7.42 μg MC g^{-1} fw	China	Chen et al. (2005)
	Digestive tract	4.54 μg MC g^{-1} fw		
	Gonad	2.62 μg MC g^{-1} fw		
	Foot	0.06 μg MC g^{-1} fw		
Anodonta woodiana, *Hyriopsis cumingii*,	Intestine	20.65 μg MC g^{-1} dw	China	Chen and Xie (2005a)
Cristaria plicata,	Hepatopancreas	38.48 μg MC g^{-1} dw		
Lamprotula leai Freshwater mussels	Visceral mass	1.7 μg MC g^{-1} dw		
	Gills	0.64 μg MC g^{-1} dw		
	Foot	0.58 μg MC g^{-1} dw		
Mussels (species N/R)	N/R	16.0 μg MC* g^{-1} fw	Portugal	Vasconcelos (1999)
Mussels (species N/R)	Whole	2.500 μg NOD g^{-1} fw	Australia	Van Buynder et al. (2001)
Dreissena polymorpha Zebra mussel	Whole	30 μg MC g^{-1} afdw	Netherlands	Ibelings et al. (2005)
Mytilus edulis Blue mussel	Whole	2.15 μg NOD* g^{-1} dw	Baltic Sea	Sipiä et al. (2001b)
Mytilus edulis Blue mussel	Whole	1.49 μg NOD g^{-1} dw	Baltic Sea	Sipiä et al. (2002)
Macoma balthica Clam	Whole	0.13 μg NOD g^{-1} dw	Baltic Sea	Sipiä et al. (2002)
Anodonta grandis simpsoniana Giant floater mussel	Whole	1.35 μg MC g^{-1} dw	Canada	Prepas et al. (1997)
Unio douglasiae	Hepatopancreas	420 μg MC g^{-1} dw	Japan	Yokoyama and Park (2002)
Cristaria plicata Cockscomb mussel	Hepatopancreas	297 μg MC g^{-1} dw	Japan	Yokoyama and Park (2002)
Lymnaea stagnalis Freshwater snail	Whole	140 μg MC g^{-1} dw	Canada	Zurawell et al. (1999)
Crustacean				
Palaemon modestu,	Hepatopancreas	8.40 μg MC g^{-1} dw	China	Chen and Xie (2005b)
Macrobrachium nipponensis	Muscle	0.53 μg MC g^{-1} dw		
Freshwater shrimp	Stomach	12.42 μg MC g^{-1} dw		
Shrimp (species N/R)	Muscle	~0.010 μg MC* g^{-1} fw	Brazil	Magalhães et al. (2003)
Crayfish (species N/R)	N/R	2.7 μg MC* g^{-1} fw	Portugal	Vasconcelos (1999)
Procambarus clarkii Signal crayfish	Hepatopancreas	0.08 μg MC g^{-1} dw	China	Chen and Xie (2005b)
	Muscle	0.05 μg MC g^{-1} dw		
	Stomach	9.97 μg MC g^{-1} dw		
Prawn (species N/R)	Muscle	0.022 μg NOD g^{-1} fw	Australia	Van Buynder et al. (2001)
	Viscera	6.400 μg NOD g^{-1} fw		
Crab (species N/R)	Muscle	0.103 μg MC* g^{-1} fw	Brazil	Magalhães et al. (2003)
Fish				
Tilapia rendalli Redbreast tilapia	Liver	31.1 μg MC g^{-1} fw	Brazil	Magalhães et al. (2001)
	Viscera	67.8 μg MC g^{-1} fw		
	Muscle	0.026 μg MC g^{-1} fw		
Carassius auratus Red cap oranda	Muscle	3.19 μg MC g^{-1} dw	China	Xie et al. (2005)
Fish (species N/R)	Muscle	0.039 μg MC* g^{-1} fw	Brazil	Magalhães et al. (2003)
Odontesthes bonariensis Silverside	Liver	1.01 μg MC g^{-1} fw	Argentina	Cazenave et al. (2005)
	Gills	0.10 μg MC g^{-1} fw		
	Muscle	0.34 μg MC g^{-1} fw		
Osmerus eperlanus Smelt	Liver	874 μg MC g^{-1} afdw	Netherlands	Ibelings et al. (2005)
Gymnocephalus cernua Ruffe	Liver	194 μg MC g^{-1} afdw		
Perca fluviatilis Perch	Liver	51 μg MC g^{-1} afdw		
Cyprinus sp. Carp	Muscle	0.3 μg MC* g^{-1} fw	Portugal	Vasconcelos (1999)
Barbus sp. Barbel	Muscle	0.1 μg MC* g^{-1} fw		
Lisa sp. Grey mullet	Muscle	0.1 μg MC* g^{-1} fw		
Platichthys flesus Flounder	Liver	0.14 μg NOD g^{-1} dw	Baltic Sea	Sipiä et al. (2001a)
Gadus morhua Cod	Liver	0.05 μg NOD g^{-1} dw	Baltic Sea	Sipiä et al. (2001a)
Platichthys flesus Flounder	Liver	0.41 μg NOD g^{-1} dw	Baltic Sea	Sipiä et al. (2002)
Platichthys flesus Flounder	Liver	0.637 μg NOD g^{-1} fw	Baltic Sea	Karlsson et al. (2003)
Platichthys flesus Flounder	Liver	0.399 μg NOD* g^{-1} dw	Baltic Sea	Sipiä et al. (2001b)
Flounder, Snapper, Tailor,	Muscle	0.003 μg NOD g^{-1} fw	Australia	Van Buynder et al. (2001)
Garfish (species N/R)	Viscera	0.152 μg NOD g^{-1} fw		

Only free MC concentrations are reported. "*" indicates when ELISA results were not confirmed by another method.
MC were summed when reported as variants. dw = Dry weight, fw = fresh weight, afdw = ash free dry weight, N/R = not reported.

compared to the more-readily consumed eukaryotes (Haney, 1987; Lampert, 1987). Cyanobacteria possess the capability to store abundant nutrients (e.g., phosphorus) and some species can convert gaseous nitrogen to ammonia via nitrogen fixation; both features afford cyanobacteria more uniform access to limiting nutrients compared to eukaryotic microalgae (Stewart, 1967). As compared to eukaryotes, the structure of the cyanobacterial cell wall allows greater hyper-osmolarity, reducing energy expenditure for active solute transport (Raven, 2003). The presence of gas vacuoles allows cyanobacterial cells to adjust their vertical position in the water column, either by floating toward light sources or sinking to deeper waters to access higher nutrient concentrations or avoid photo-oxidative damage (Walsby and Booker, 1980; Klemer et al., 1996). Cyanobacteria, unlike most autotrophs, can directly absorb and utilize bicarbonate in addition to CO_2 (Shapiro, 1997), a competitive advantage under conditions of increased photosynthesis and depleted CO_2 concentrations (i.e., basic conditions).

Cyanobacteria are also well adapted to high-light and low-light conditions. Cyanobacteria benefit from photosynthetic reaction centers complemented by phycobilisomes, protein complexes containing three water soluble accessory pigments (phycoerythrin, allophycocyanin, and phycocyanin), which increase the light energy available for photosynthesis and growth (Goodwin and Mercer, 1983). Additionally, cyanobacteria can alter the number and protein structure of their photosystems in the short term, and in the long term, induce gene and *de novo* synthesis of photosynthetic proteins, allowing positive growth under extremely low-light environments and avoidance of photo-oxidative damage under high-light conditions (Bhaya et al., 2000; Kirilovsky, 2007).

Cyanobacteria are well known for their ability to produce a large number of diverse secondary metabolites, compounds that are not essential for primary cell metabolism (Vining, 1992). In this review we will summarize cyanobacterial secondary metabolites that (1) result in undesirable tastes and odors (odorous metabolites) or (2) are biochemically active (bioactive metabolites) in marine and freshwater extensive and intensive aquaculture (Fig. 1). For the scope of this paper, biochemically active metabolites include compounds that can cause mortality, initiate or promote tumors, or deteriorate the health of cultivated species or their prey species by affecting feeding, growth, or immune defense. Toxic metabolites may also have the potential to harm consumers if they accumulate in the product. We will begin by describing exposure pathways for aquaculture organisms, and in the end, make suggestions as to the future management of intensive and extensive systems to minimize impacts from cyanobacterial metabolites.

2. Exposure pathways in aquaculture

2.1. Aquatic food web

Aquaculture species are exposed to odorous and bioactive secondary metabolites through the ingestion of cyanobacteria, consumption of contaminated food items (e.g., prey or detritus), and/or absorption of dissolved compounds from the water column (e.g., after leakage from cells or cell lysis). Ingestion of cyanobacteria can be accidental or intentional, with accidental ingestion more likely to occur in intensive systems in the presence of a dense bloom. Catfish, for example, ingest surface scum while feeding on floating food pellets, thereby increasing their potential exposure to cyanobacterial metabolites (Zimba et al., 2001b). Planktivorous fish, such as carp and tilapia, intentionally ingest cyanobacteria; however, high densities of toxic cyanobacteria significantly reduce grazing rates, potentially reducing the growth rate of these cultured species (Beveridge et al., 1993; Keshavanath et al., 1994). Dissolved metabolites, such as off-flavor compounds, are transported across gill and carapace/skin membranes; however, due to the relatively hydrophilic nature of many of the toxic metabolites, this pathway is thought to account for less exposure than direct ingestion of cyanobacteria.

Aquaculture species also are exposed to cyanobacterial metabolites through the aquatic food web. Metabolites accumulate in the tissue of the consumer (e.g., hepatotoxins, cytotoxins, neurotoxins, dermatoxins, odorous metabolites) facilitating transfer through the food chain. Hepatotoxins were transferred to Atlantic salmon through the consumption of toxin-containing biofouling organisms and crab larvae, resulting in net-pen liver disease (Andersen et al., 1993; Kent et al., 1996). Trophic transfer also has been demonstrated under laboratory conditions in which hepatotoxins were transferred from zooplankton to fish (Engström-Öst et al., 2002; Karjalainen et al., 2005; Smith and Haney, 2006). Other potential vectors include aquatic plants and macroalgae (Pflugmacher et al., 1999, 2007) and other benthic and pelagic animals that accumulate cyanobacterial metabolites, such as snails, crayfish, fish, and shrimp (Table 1).

2.2. Human exposure

Humans can be exposed to bioactive secondary metabolites through the consumption of laden tissue (Sections 4.1–4.3) or through dermal or respiratory contact during maintenance and harvesting (Sections 4.4 and 4.5). Based on regional or ethnic food preferences, various organs of aquatic animals are considered edible, including the foot, gonads, or whole body of gastropods, the foot or whole body of

Table 2
Maximum accumulation of cyanobacterial toxins in the tissues of aquatic animals in aquaculture systems and any reported effects

Organism	Source(s)	Ambient toxin particulate+dissolved	Tissue	Maximum tissue load	Reported effects	Ref
Crustacean						
Litopenaeus vannamei White shrimp	*Microcystis aeruginosa*, *Anabaena*	45 μg MC L^{-1}	Hepatopancreas	55 μg MC g^{-1} fw	Mortality, liver lesions, hepatocyte degeneration	1
Penaeus monodon	*Microcystis*, *Oscillatoria*	1.2 mg TEH kg^{-1} dw	Hepatopancreas	80 μg TEH g^{-1} dw	N/R	2
Black tiger prawns	*N. spumigena*	2000 mg NOD kg^{-1} dw	N/R	N/R	Mortality	2
Cherax qudricarinatus	*C. raciborskii*	45 μg CYL L^{-1}	Hepatopancreas	4.3 μg CYL g^{-1} dw	No histological abnormalities	3
Redclaw crayfish			Muscle	0.9 μg CYL g^{-1} dw		
Fish						
Ictalurus punctatus	*M. aeruginosa*	78 μg MC L^{-1}	Liver	250 μg MC g^{-1} fw	Mortality, swollen hepatopancreatic tissue	4
Catfish						
Oreochromis niloticus	*M. aeruginosa*	1120 mg MC kg^{-1} dw	Gut	0.82 μg MC g^{-1} fw	N/R	5
Nile tilapia		~7504 μg MC L^{-1}	Liver	0.53 μg MC g^{-1} fw	N/R	
			Kidney	0.40 μg MC g^{-1} fw	N/R	
			Muscle	0.10 μg MC g^{-1} fw	N/R	
Rainbow fish	*C. raciborskii*	45 μg CYL L^{-1}	Viscera	1.2 μg CYL g^{-1} dw	N/R	3

Only free MC concentrations are reported. N/R = Not reported. Toxins: MC = microcystins, NOD = nodularin, TEH = total extractable hepatotoxin by ELISA, CYL = cylindrospermopsin. References: (1) Zimba et al. (2006), (2) Kankaanpää et al. (2005), (3) Saker and Eaglesham (1999), (4) Zimba et al. (2001b), (5) Mohamed et al. (2003).

Table 3
Summary of laboratory experiments with freshwater and marine animals immersed in toxic cyanobacteria

Organism	Cyanobacteria concentration	Ambient toxin concentration	Exposure design	Detection method	Maximum whole body load	Maximum tissue load	Reference
Mollusk							
Dreissina polymorpha Zebra mussel	*Microcystis aeruginosa* 3.8 mg dw L^{-1}	11.8 μg MC L^{-1}	Daily delivery for 3 weeks	LC-MS	11 μg MC g^{-1} dw	N/R	Dionisio Pires et al. (2004)
Unio douglasiae	*M. ichthyoblabe* 14.5 mg dw L^{-1}	50 μg MC L^{-1}	Every 2 days for 15 days	HPLC	N/R	630 μg MC g^{-1} dw hepatopancreas	Yokoyama and Park (2003)
Mytilus galloprovincialis Bay mussel	*M. aeruginosa* 10^8 cells L^{-1}	2.5 μg MC L^{-1}	Daily delivery for 4 days	ELISA	10.7 μg MC g^{-1} dw	N/R	Vasconcelos (1999)
Mytilus galloprovincialis Bay mussel	*M. aeruginosa* 10^8 cells L^{-1}	7.7 μg MC L^{-1}	Daily delivery for 16 days	HPLC	10.5 μg MC g^{-1} dw	N/R	Vasconcelos (1995)
Mytilus edulis Blue mussel	*M. aeruginosa* 100 mg dw L^{-1}	305 μg MC L^{-1}	One time delivery, then 3 days	PPIA	1.1 μg MC g^{-1} dw*	N/R	Williams et al. (1997)
Mytilus edulis Blue mussel	*Nodularia spumigena* 50 μg chl *a* L^{-1}	15.6 μg NOD L^{-1}	One time delivery for 12 h	LC-ESI-MS	13.8 μg NOD g^{-1} dw	N/R	Strogyloudia et al. (2006)
Anodonta cygnea Swan mussel	*Oscillatoria agardhii* 10–20 mg dw L^{-1}	40–60 μg MC L^{-1}	Constant delivery for 15 days	HPLC	72 μg MC g^{-1} dw	130 μg MC g^{-1} dw hepatopancreas	Eriksson et al. (1989)
Anodonta cygnea Swan mussel	*Aphanizomenon issatschenkoi* 1.4×10^9 cells L^{-1}	25.5 nmol PSP L^{-1}	Daily delivery for 14 days	HPLC	0.3 μg PSP g^{-1} fw	N/R	Pereira et al. (2004)
Stylocheilus striatus, *Bursatella leachii*, *Diniatys dentifer*	*Lyngbya majuscula*	≤40.7 μg LA g^{-1}, ≤0.4 μg DAT g^{-1} ww	One time delivery, then 10 days	LC-MS/MS	3.9 μg LA g^{-1} fw	6.3 μg LA g^{-1}, fw 5.3 μg DAT g^{-1} fw digestive gland	Capper et al. (2005) (loads estimated from ref.)
Crustacean							
Cherax quadricarinatus Redclaw crayfish	*Cylindrospermopsis raciborskii* 0.5–2.0×10^6 cells L^{-1}	111 μg CYL L^{-1}	One time delivery of sonicated cells or culture, then 14 days	LC-MS	N/R	0.54 μg CYL g^{-1} dw hepatopancreas 0.12 μg CYL g^{-1} dw muscle	Saker and Eaglesham (1999)
Fish							
Hypophthalmichthys molitrix Silver Carp	*Microcystis viridis* 366 μg chl *a* L^{-1}	628 μg MC g^{-1} dw	One time delivery and then exposure for 10 days	HPLC	N/R	17.8 μg MC g^{-1} dw liver 1.77 μg MC g^{-1} dw muscle	Xie et al. (2004)
Cyprinus carpio L. Common carp	*Anabeana* sp. 10^{10} cells L^{-1}	1170 μg ANA L^{-1}	Daily delivery for 4 days	HPLC	0.768 μg ANA g^{-1} dw	N/R	Osswald et al. (2007)

N/R = Not reported. "*" indicates when PPIA results were not confirmed by another method. ESI = electrospray ionization. Toxins: MC = microcystins, NOD = nodularin, PSP = paralytic shellfish poisoning toxins, ANA = anatoxin, CYL = cylindrospermopsin, LA = lyngbyatoxins, DAT = debromoaplysiatoxin.

bivalves, the anterior tissue (with or without the digestive tract) or whole body of shrimp/prawns, soft tissue of crayfish, and the muscle tissue or whole body of fish. All of these organs accumulated bioactive metabolites under natural conditions (Table 1). The World Health Organization published a tolerable daily intake guideline (TDI) for human exposure to the potent hepatotoxin, microcystin-LR (Section 4.1). Nine out of the 19 studies summarized in Tables 1 and 2 reported hepatotoxin accumulation in edible tissues that exceeded the TDI, (0.04 μg kg^{-1} bw d^{-1} assuming 100–300 g fw tissue consumed), suggesting that human intoxication is possible through both intensive and extensive aquaculture. Pond water and farmed animals are occasionally analyzed for cyanobacterial toxins during or immediately after a die-off event (Zimba et al., 2001b, 2006), but rarely does regular monitoring for accumulated toxins occur. Regular low-frequency monitoring could prevent additional product processing, maintenance, or loss (Fig. 1) as (1) ambient toxin concentrations can vary over a season (Kankaanpää et al., 2005) and (2) accumulation or toxicosis occurs at sub-lethal concentrations (Andersen et al., 1993; Kent et al., 1996; Mohamed et al., 2003). Exposure guidelines have not been estimated for other bioactive metabolites due to insufficient data. Farmers can experience dermatitis, blisters, or necrosis after exposure to dermatoxins (Section 4.4) and anecdotal reports associate irritant toxins with digestive and respiratory distress (Section 4.5).

Aquatic organisms are generally considered more tolerant of cyanobacterial toxins than mammals as a result of their coevolutionary history, which can reduce the likelihood for catastrophic losses of the cultured species but increase the potential for human exposure to the toxins. Evidence suggests that gastropods, bivalves and crayfish provide a greater risk than fish to human consumers as they accumulate higher concentrations of cyanobacterial toxins (Tables 1 and 2), are relatively more tolerant of the toxins, and are frequently consumed whole. Planktivorous tilapia and shrimp/prawns are also relatively tolerant of cyanobacterial toxins, and therefore, accumulate high concentrations in their liver/hepatopancreas in intensive aquaculture facilities and natural systems (Tables 1 and 2). Free toxin concentrations in fish and shrimp/prawn muscle, however, are generally an order of magnitude less than concentrations detected in the liver/hepatopancreas. Although this suggests that human intoxication is less likely if only fish or shrimp/prawn muscle is consumed, Van Buynder et al. (2001) showed that boiling of whole shrimp (e.g., such as for soup) redistributed hepatotoxins from the viscera to muscle tissue. The mechanism behind this redistribution is unknown. Additionally, accumulation in aquaculture species can occur quickly after exposure to toxic cyanobacteria, as exemplified in numerous immersion laboratory experiments (Table 3), showing that even short term blooms (i.e., days to weeks) should be of concern if high cell densities or cyanobacterial toxins are present.

3. Odorous secondary metabolites

The term "off-flavor" is used to describe the accumulation of odorous compounds within water or tissue produced from biological origins. Over 200 such compounds have been identified as being produced by algae (Watson, 2003). In the case of potable water, consumer perception of the quality of drinking water is typically influenced by odor and taste (Davies et al., 2004). While Ames test

Table 4
Cyanobacteria species known to produce off-flavor compounds

Source	Odorous metabolite(s)	Reference
Anabaena circinalis Kütz. Rabenhorst *ex* Bornet & Flahault	Geosmin	Henley (1970)
A. crassa Lemmermann Komarkova-Legenerova & Cronberg	Geosmin	Watson (2003)
A. laxa	Geosmin	Rashash et al. (1995)
A. lemmermanii Richter	Geosmin	Watson (2003)
A. macrospora Klebahn	Geosmin	Matsumoto and Tsuchiya (1988)
A. solitaria Klebahn	Geosmin	"
A. viguieri Denis & Frémy	Geosmin	Persson (1988)
Aphanizomenon flos-aquae (Linnaeus) Ralfs	Geosmin	Jüttner et al. (1986)
Aphan. gracile Lemmermann	Geosmin	Jüttner et al. (1986)
Calothrix parietina	Ketones, ionines	Höckelmann and Jüttner (2005)
Fisheriella muscicola (Gomont)	Geosmin	Wu and Jüttner (1988)
Geitlerinema spendidum (Agardh *ex* Gomont) Anagnositidis & Komárek (=*Oscillatoria splendida*)	Geosmin	Tabachek and Yurkowski (1976)
Hyella sp.	MIB	Izaguirre and Taylor (1995)
Jagerinema genimatum (Meneghini *ex* Gomont) Anagnositidis & Komárek (=*Oscillatoria geminata*)	MIB	Matsumoto and Tsuchiya (1988)
Leibleinia subtilis (Holden) Anagnositidis & Komárek (=*Lyngbya subtilis*)	Geosmin	Schrader and Blevins (1993)
Lyngbya aestuarii Lieberman	Geosmin	Tabachek and Yurkowski (1976)
L. cryptovaginata	Geosmin	Cited in Jüttner and Watson (2007)
L. wollei (Farlow *ex* Gomont)	MIB	Zimba, pers. obs.
Oscillatoria amphibia (C. Aghardh *ex* Gomont)	Geosmin	Cited in Jüttner and Watson (2007)
O. curiceps C. Aghardh	MIB	Izaguirre et al. (1982)
O. limosa C. Aghardh	MIB	Izaguirre and Taylor (1995)
O. tenuis Gardiner	MIB	Izaguirre et al. (1982)
O. variabilis Rao	MIB	Tabachek and Yurkowski (1976)
Phormidium amoeneum (Kützing) Anagnositidis & Komárek (=*O. amoena*)	Geosmin	Tsuchiya et al. (1981)
Phorm. autumnale (Agardh) Trevisan *ex* Gomont	MIB	Zimba, pers. obs.
Phorm. breve (Gomont) Anagnositidis & Komárek (=*O. brevis*)	Geosmin, MIB	Naes et al. (1988)
Phorm. calcicola Gardner	Geosmin, MIB	Cited in Jüttner and Watson (2007)
Phorm. cortianum (Meneghini) Anagnositidis & Komárek (=*O. cortiana*)	Geosmin	Tabachek and Yurakowski (1976)
Phorm. formosum (Bory *ex* Gomont) (=*O. formosa*)	Geosmin	Persson (1988)
Phorm. favosum (Bory) Gomont	MIB	Sugiura et al. (1998)
Phorm. simplissimum (Gomont) Anagnositidis & Komárek (=*O. simplicissima*)	Geosmin	Persson (1988)
Phorm. tenue (C. Aghardh *ex* Gomont) Anagnositidis & Komárek (=*O. amoena*)	MIB	Persson (1988)
Phorm. tenue=*P. tergestinum* (Kutz.)		
Phorm. uncinatim (C. Aghardh) Gomont	Geosmin	Sugiura et al. (1998)
Phorm. viscosum Kütz.	Geosmin	"
Phorm. sp.	Geosmin, MIB	Zimmerman et al. (1995); Berglind et al. (1983)
Planktothrix aghardhii (Gomont) Anagnositidis & Komárek (=*O. aghardhii*)	Geosmin, MIB	Berglind et al. (1983)
Plankto. cryptovaginata (Schkorbatow) Anagnositidis & Komárek	MIB	Persson (1988)
Plankto. perornata f. *attenuata* (Skuja) Anagnositidis & Komárek (syn. *O. chalybea*)	MIB	van der Ploeg et al. (1995)
Plankto. prolifica (Greville *ex* Gomont) Anagnositidis & Komárek	Geosmin, MIB	Berglind et al. (1983)
Porphyrosiphon martensianus (Meneghini *ex* Gomont) Anagnositidis & Komárek	MIB	Izaguirre and Taylor (1995)
Pseudanabaena articulata Skuja	MIB	Zimba et al. (1999)
Pseudo. catenata Lauterborn	Geomin, MIB	Izaguirre et al. (1999)
Pseudo. limnetica (Lemmermann) Komarék (=*Oscillatoria limnetica*)	MIB	Matsumoto and Tsuchiya (1988)
Rivularia sp.	Ketones, ionones	Höckelmann and Jüttner (2005)
Schizothrix muellerii Nägeli	Geosmin	Kikuchi et al. (1973)
Symploca muscorum (C. Aghardh) Gomont	Geosmin	Tabacheck and Yurkowski (1976)
Synechococcus cedrorum Sauvageau	MIB?	
Synech. sp.	MIB	Zimba, pers. obs.
Tychonema bornetii (Zukal) Anagnositidis & Komárek	Geosmin	Berglind et al. (1983)
Tycho. granulatum (*Gardner*) Anagnositidis & Komárek	Geosmin, MIB	Matsumoto and Tsuchiya (1988)
Tolypothrix distorta	Ketones, ionones	Höckelmann and Jüttner (2005)

Listing includes historical name (and current taxonomic identification sensu Komárek et al., 2003), as well as initial publication referenced therein.

results have conclusively shown no mutagenic effects of the off-flavor compound geosmin (Dionigi et al., 1993), consumers purchasing farm-raised aquaculture products (or potable water) expect uniform quality, with no disagreeable taste/odor compounds present (Tucker, 2000).

The presence of objectionable odors is considered to be a major impediment to growth of the freshwater aquaculture industry, as orderly grow-out and sale of fish is compromised (Dionigi et al., 1998). Within the US channel catfish industry, it is estimated that over 30% of the potential revenue is lost from off-flavor contamination. Costs associated with off-flavor would include holding and feeding fish until off-flavors decrease, delays in restocking ponds until fish are acceptable for sale/harvest, as well as fish mortalities resulting from predation, disease, or toxic events in ponds (Engle et al., 1995). Off-flavors have been reported to impact a number of commercially important species, including Nile tilapia, *Oreochromis niloticus* (Yamprayoon and Noomhorn, 2000), shrimp (Whitfield et al., 1988), Atlantic salmon, *Salmo salar* (Farmer et al., 1995), rainbow trout, *Salmo gairdneri* (From and Horlyck, 1984), catfish species (Lovell et al., 1986; Martin et al., 1987) cultured largemouth bass, *Micropterus salmoides*, and white sturgeon, *Acipenser transmontanus* (Schrader et al., 2005). Additionally, unpublished impacts on lobster, *Homarus americanus*, have been documented in holding tanks (Zimba and Grimm, pers. comm.).

A recent review of off-flavor producing algae and cyanobacteria (Watson, 2003) provides a thorough listing of known off-flavor producing algal species — this list is updated herein (Table 4). Cyanobacteria species, particularly filamentous forms produce more than 25% of all known off-flavor compounds. Geosmin (E1, 10-dimethyl-E-9-decalol) and 2-methylisoborneol (MIB) typically account for the majority of reported off-flavor odors. Strong evidence for two new off-flavor compounds from cyanobacteria was recently presented; the new odorous metabolites include (1) hydroxyketones formed via fermentation pathways, and (2) norcarotenoids (e.g., β-cyclocitral), resulting from the degradation of carotenoids (Höckelmann and Jüttner, 2005). The latter compound had been previously identified in field samples of coccoid cyanobacteria, particularly *Microcystis* sp. (Zimba and Grimm, 2003). Höckelmann and Jüttner (2005) conclusively showed that cyanobacteria were responsible for their synthesis.

Classic methods for analyzing water and fish tissue include closed loop stripping methods (Johnsen and Kuan, 1987). The development of solid-phase extraction methodology for both water (Lloyd et al., 1998) and fish samples (Grimm et al., 2000) has facilitated increased sample throughput thereby increasing replication and precision in larger scale studies than previously possible (Zimba et al., 2001a; Grimm and Zimba, 2005; Zimba and Grimm, 2003). This analytical approach was recently used to revise threshold concentrations of MIB and geosmin downward by 50% (MIB=10–20 ppt, geosmin=25–50 ppt) relative to trained flavor checkers analyzing paired samples (Grimm et al., 2004).

4. Bioactive secondary metabolites

Cyanobacteria produce many bioactive compounds that are structurally and biochemically diverse. Here we group the secondary metabolites based upon their potential activity against aquaculture species, whether the metabolite may be active towards a target enzyme, cell type, organ, or an animal's behavior.

4.1. Hepatotoxins

4.1.1. Structure and source

Cyanobacterial hepatotoxins include more than 70 variants of microcystins (Zurawell et al., 2005, and references therein) and eight variants of nodularins (Mazur-Marzec et al., 2006). Microcystins are produced worldwide by species of *Microcystis*, *Anabaena*, *Nostoc*, and *Oscillatoria*/*Planktothrix*, while nodularins are produced by species of *Nodularia*. Microcystins and nodularins are monocyclic heptapeptides and pentapeptides, respectively, with both toxins possessing the unique amino acid Adda ((2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid). Primary microcystin congeners are named according to their substitutions at the two variable positions (e.g., microcystin-LR, MC-LR, contains amino acids leucine and arginine).

4.1.2. Mode of action/detoxification

Ingested microcystins and nodularins are absorbed into the blood across the ileum, gill, or lung membranes and preferentially accumulate in hepatocytes (i.e., liver cells) as a result of the cells' abundant organic anion transporting polypeptides, OATPs, also known as the bile acid transport system (Runnegar et al., 1995; Fischer et al., 2005). OATPs are responsible for the active membrane transport of bile acids, steroids, and various peptides (Hagenbuch and Meier, 2003). Uptake of hepatotoxins into other organs is constrained by the specificity of the OATPs present and the overall hydrophilic nature of the molecule (Fischer et al., 2005). It is possible that a few non-polar variants of microcystins can passively diffuse across membranes; however, this has not yet been confirmed (Kuiper-Goodman et al., 1999). Hepatotoxins have also accumulated in kidney, intestine, and skeletal muscle of mammals (Ito et al., 2001) and fish (Tables 2 and 3) and within the body muscle, gut, gonads, heart, and foot tissues of aquatic invertebrates (Van Buynder et al., 2001; Chen et al., 2005a; Chen and Xie 2005a,b; Kankaanpää et al., 2005) suggesting the OATPs present in these organs accommodate hepatotoxin transport; however, the hepatotoxin specificity of the OATPs have only been confirmed in liver and brain cells (Fischer et al., 2005; Meier-Abt et al., 2007).

Once inside cells, microcystins and nodularins inhibit protein phosphatases 1 and 2A, leading to an imbalance in phosphorylation/dephosphorylation reactions and eventual loss of cell structure (Runnegar and Falconer 1986; Carbis et al., 1996), oxidative stress (Ding et al., 2001), tumor promotion (Nishiwaki-Matsushima et al., 1991), or in the case of nodularin, tumor initiation (Ohta et al., 1994). Initial inhibition of phosphatase activity is through reversible reactions between the hepatotoxins and protein phosphatases. Microcystins subsequently undergo a secondary reaction with the target enzyme, forming an irreversible, covalent linkage through its Mdha residue (*N*-methyldehydroalanine). Microcystins in the first reversible reaction (i.e., non-covalently-bound or free microcystins) are typically quantified in animal tissue, as this form is hypothesized to be more bioavailable and can be quantified via many methods. The covalently-bound portion is estimated by subtracting the non-covalently-bound portion from the total tissue load as determined by tissue oxidation (Ott and Carmichael, 2006). Little is known in regards to the bioavailability of covalently-bound microcystins. Both microcystins and nodularins cause oxidative stress by increasing the formation of harmful reactive oxygen species (ROS) and/or decreasing the antioxidant capability of the cell (e.g., inhibiting detoxification enzymes), resulting in DNA degradation and/or cell death (i.e., apoptosis and necrosis). More specifically, microcystins cause an increase in ROS production, antioxidant enzyme activity, lipid peroxidation, cell death, and DNA damage (Ding et al., 2001; Pinho et al., 2003, 2005; Zegura et al., 2004, 2006; Komatsu et al., 2007) and nodularins decrease the activity of glutathione peroxidase, superoxide dismutase, and catalase (Lankoff et al., 2002). Recent evidence suggests that other enzymes may be a target for hepatotoxins, including mitochondrial aldehyde dehydrogenase (Chen et al., 2006), ATP-synthase (Mikhailov et al., 2003) and acetylcholinesterase (Lehtonen et al., 2003). The pathways through which tumor initiation and promotion occur are not currently known.

Microcystins and nodularins are metabolized by conjugation to glutathione (GSH) via glutathione *S*-transferase in mammals (Kondo et al., 1996), aquatic vertebrates (Pflugmacher et al., 1998; Wiegand et al., 1999), and aquatic invertebrates (Vinagre et al., 2002; Beattie et al., 2003; Chen and Xie 2005a; Pflugmacher et al., 2005).

Presumably, conjugation forms a more polar compound, leading to its rapid excretion in urine and/or feces. The microcystin–GSH conjugate and microcystin–cysteine conjugate, another biotransformation product, inhibited PP1 and PP2A but to a lesser degree than the parent toxin when intratracheally or intravenously administered to mice (Kondo et al., 1992; Ito et al., 2002b).

4.1.3. Effects/accumulation in aquatic organisms

Hepatotoxins have caused detrimental effects in aquatic vertebrates and invertebrates under laboratory and field conditions. The effects of microcystins on the embryonic, juvenile, and adult life stages of fish include histopathological damage in the liver, kidneys, gills, intestines, heart or spleen, disrupted osmoregulation, altered serum biochemistry, malformation of embryonic–larval alimentary system, reduced growth rate, induced stress response, and modified swimming behavior (Malbrouck and Kestemont, 2006, and references therein). Similarly, exposure to nodularin resulted in histopathological liver damage in sea trout, *Salmo trutta* m. *trutta* L. (Kankaanpää et al., 2002). In aquatic invertebrates, microcystin exposure disrupted osmoregulation of an estuarine crab (*Chasmagnathus granulata*) through the inhibition of Na^+, K+-ATPase activity in the anterior gills (Vinagre et al., 2002) and caused oxidative stress and histopathological damage in the crab's hepatopancreas (Pinho et al., 2003). Detailed histopathological research has not been reported for other aquaculture targets such as snails, bivalves or shrimp/prawns exposed to hepatotoxins. Instead, immersion studies have shown that freshwater and marine bivalves survived days of exposure to high concentrations of toxic cyanobacteria (10^8 cells L^{-1} or 100 mg dw L^{-1}) containing 2.5–300 μg L^{-1} of hepatotoxin (Table 3), suggesting these species are tolerant at ecologically relevant concentrations, for at least short periods of time. The signal crayfish, *Pacifastacus leniusculus*, also was tolerant of hepatotoxins as test organisms did not display altered feeding, behavior, hepatopancreas weight, or glucose levels after ingesting a total of 430 μg of microcystins (as pelletized *Oscillatoria sancta*) over 14 days (Liras et al., 1998).

Damage to the liver/hepatopancreas can be reversed if aquatic organisms are removed from the toxic environment (Kankaanpää et al., 2002); however, mortality can result if exposure continues or organisms are exposed to high doses. The hepatotoxin LD_{50} (the toxin dose that results in 50% mortality of the test population), administered to mammals by intraperitoneal injection (i.p.), ranges between 50 μg kg^{-1} (for MC-LR) and 300 μg kg^{-1}, depending on the congener (Sivonen and Jones, 1999, and references therein). Fish are generally considered more tolerant of hepatotoxins, with carp and perch LD_{50} (i.p.) values of 300–500 μg kg^{-1} and 1500 μg kg^{-1} for MC-LR, respectively (Råbergh et al., 1992; Ibelings et al., 2005). In natural systems, hepatotoxin-producing cyanobacterial blooms were associated with mass fish mortality (Rodger et al., 1994; Lindholm et al., 1999); however, other detrimental biological, chemical, and physical agents coincided with the bloom or its degradation, and therefore, the exact cause could not be determined. Direct evidence has been presented, however, for hepatotoxin-induced toxicosis and mortality in intensive aquaculture systems — specifically, pond farming of catfish and white shrimp (Zimba et al., 2001b, 2006) and net-pen farming of Atlantic salmon, chinook salmon, and rainbow trout (Andersen et al., 1993; Kent et al., 1996). These systems allowed for more definitive studies as they are relatively controlled environments in which organisms cannot flee to "uncontaminated" areas of the water body. In these studies, the observed pathology and mortality in the ponds or pens was additionally paired with laboratory experiments in which the pathology was reproduced through immersion, gavage, or i.p. injections with bloom scum, source water, bloom extract, or purified toxin.

Additionally, hepatotoxins can have indirect negative effects on extensive aquaculture productivity, where the growth of cultured species depends largely upon thriving populations of lower trophic levels (i.e., aquatic plants, macroalgae, microbes, zooplankton, microcrustaceans, and more edible or nutritious phytoplankton species). When exposed to ecologically relevant concentrations of MC-LR (0.5 μg L^{-1} and 1.0 μg L^{-1}), four species of aquatic plants and the macroalgae *Cladophora* sp. significantly reduced photosynthesis and/or growth rates (Pflugmacher, 2002). Another macroalgae, *Fucus vesiculosus*, underwent oxidative stress after exposure to nodularin-containing extract (Pflugmacher et al., 2007). The microbial food web may also suffer in the presence of a hepatotoxic cyanobacterial bloom, as exposure decreased the survival of the protozoan, *Tetrahymena pyriformis* (Ward and Codd, 1999) and reduced the growth rate of nanoflagellates (Christoffersen, 1996). In the presence of hepatotoxin-producing cyanobacteria, the zooplankton community in extensive aquaculture ponds can shift to less desirable species (e.g., species that are more evasive or are smaller than the preferred food size) in the presence of hepatotoxin-producing cyanobacteria as a result of reduced fecundity, decreased feeding rates, and increased mortality of large-bodied species (Thostrup and Christoffersen, 1999; Engström et al., 2000; Geng et al., 2006; Liu et al., 2006). Hepatotoxic cyanobacteria also have proven harmful to crustaceans, decreasing grazing rates in the amphipod, *Gammarus zaddachi*, and mysid shrimp, *Mysis mixta* (Engström et al., 2001; Korpinen et al., 2006) and causing mortality in fairy shrimp, *Thamnocephalus platyurus* (Keil et al., 2002), brine shrimp, *Artemia salina* (Metcalf et al., 2002), and the microcrustacean, *Kalliapseudes schubartii* (Montagnolli et al., 2004). Thus, hepatotoxins can limit aquaculture yield indirectly by reducing the productivity of prey (food supply) or altering the community structure towards less nutritious prey species.

Hepatotoxins accumulate in pelagic and benthic animals in both natural (Table 1) and aquaculture systems (Table 2) throughout the world. As the liver/hepatopancreas is a major accumulator of the hepatotoxins, and the muscle tissue is coveted for human consumption, these two organs are most commonly analyzed for free hepatotoxins (i.e., the non-covalently-bound portion). While microcystin concentrations in fish muscle have been as high as 0.10 μg MC g^{-1} fw in aquaculture (Mohamed et al., 2003) and 0.34 μg MC g^{-1} fw in natural systems (Cazenave et al., 2005), nodularin has only been confirmed in muscle tissue in 1 out of 4 studies in which wild fish were analyzed (0.0025 μg NOD g^{-1} fw, Table 1). Similarly, maximum nodularin concentrations in fish liver (0.637 μg NOD g^{-1} fw) and bivalves (2.5 μg NOD g^{-1} fw) have been relatively lower than reported microcystin concentrations (31 μg MC g^{-1} fw and 16 μg MC g^{-1} fw, respectively). However, it is important to point out that (1) there has been less sample effort put forth towards nodularin detection in natural systems, (2) microcystin quantification by LC-MS or HPLC commonly includes the summation of all microcystin or nodularin variants, leaving out valuable information regarding their relative toxicities, and (3) hepatotoxin activity is often reported as microcystin-LR or nodularin equivalents after quantification via ELISA (enzyme-linked immunosorbent assay) or PPIA (protein phosphatase inhibition assay) but the actual toxin responsible was not confirmed (i.e., confirmation by LC-MS, MALDI, or HPLC is advantageous).

4.2. Cytotoxins

4.2.1. Structure and source

Cylindrospermopsin, a cyclic guanidine alkaloid, is produced by at least eight freshwater, filamentous cyanobacterial species, including *Cylindrospermopsis raciborskii*, *Umezakia natans*, *Anabaena bergeii*, *Aphanizomenon flos-aquae*, *Aphanizomenon issatschenkoi*, *Aphanizomenon ovalisporum*, *Raphidiopsis curvata*, and *Lyngbya wollei* (Falconer and Humpage, 2006, and references therein). *C. raciborskii* is the most studied species, with its distribution extending into North and South America, Asia, Australia, Africa and Europe.

Additional metabolites have been isolated from *Lyngbya* sp. which display cytotoxicity against cancer cell lines (Tan, 2007, and references therein). These compounds have not yet been shown to exhibit toxicity towards aquatic organisms, including brine shrimp or fish, and therefore, were left out of this review.

4.2.2. Mode of action/detoxification

Cylindrospermopsin (CYL) and its analogs, deoxycylindrospermopsin (Norris et al., 2001) and 7-epi-cylindospermopsin (Banker et al., 2001), have been classified as hepatotoxins as exposure causes the greatest damage to the liver/hepatopancreas; however, because effects are commonly observed in other organs as well, CYL and its analogs have also been generally classified as cytotoxins (i.e., substances toxic to cells). Other organs affected include kidneys, lungs, heart, stomach, and adrenal glands. The mechanism for cellular uptake is unknown, but it does require the bile acid transport system (Chong et al., 2002). Diffusion was suggested as a possible uptake mechanism (Chong et al., 2002), however, the charged nature of CYL makes this route unlikely without the help from a pore or transporter molecule. Cylindrospermopsin exposure results in the irreversible inhibition of protein synthesis, cellular necrosis, atrophy, DNA fragmentation, and tumor initiation or mutagenesis (Wiegand and Pflugmacher, 2005; van Apledoorn et al., 2007, and references therein). The parent compound and two analogs exhibit similar potency levels (Banker et al., 2001; Neumann et al., 2007). Long term (from 1 to 5 days) exposure to cylindrospermopsin decreased mouse i.p. LD_{50} values by ten-fold, suggesting an additive response (Ohtani et al., 1992). The detoxification pathway for CYL is unknown, but biotransformation by cytochrome *P450* is required for cytotoxicity (Froscio et al., 2003).

4.2.3. Effects/accumulation in aquatic organisms

While the mode of toxicity for CYL has been thoroughly investigated in mammals, a more organismal approach has been taken when investigating the effects in aquatic vertebrates and invertebrates. Purified CYL caused mortality of the brine shrimp, *A. salina*, giving a 48-hour LC_{50} (toxin concentration that resulted in 50% mortality of the test population in 48 h) of 2860 μg L^{-1} (Metcalf et al., 2002). Whole cells of *C. raciborskii*, containing 200–232 μg CYL L^{-1}, were toxic to the tadpole *Bufo marinus* (White et al., 2007) and snail embryos, *Melanoides tuberculata* (Kinnear et al., 2007), decreasing motility and/or survival and accumulating in tadpoles (895 μg kg^{-1} fw). The survival, growth rates, and behavior of adult snails were unaffected. In both studies, previously-lysed cell treatments were less toxic than whole cell treatments, suggesting that toxicosis is facilitated through ingestion of whole cells as opposed to the absorption of free toxin. Crayfish (*Cherax quadricarinatus*) accumulated more CYL in their hepatopancreas (4.3 μg g^{-1} dw) and abdominal muscle tissue (0.9 μg g^{-1} dw) when exposed to a natural population of *C. raciborskii* in an aquaculture pond than when exposed to a laboratory culture for 14 days (Tables 2 and 3). No histological abnormalities were identified in exposed crayfish (Saker and Eaglesham, 1999). Rainbow fish, also collected from the aquaculture pond, accumulated 1.2 μg g^{-1} dw in viscera tissue. After 16 days of exposure, the freshwater mussel *Anodonta cygnea* accumulated up to 2.52 μg CYL g^{-1} dw, 50% of which was retained in tissues after two weeks of depuration (Saker et al., 2004). From these studies, two preliminary conclusions emerge that should be further investigated, as they can have implications for aquaculture: (1) younger life stages are more sensitive to CYL exposure than adults and (2) species that graze on cyanobacteria (e.g., crayfish, freshwater mussels, and adult snails) appear tolerant of CYL. The accumulation and possible effects of CYL and its analogs on other aquaculture species, including fish and shrimp, should also be a focus of future studies.

4.3. Neurotoxins

4.3.1. Structure and source

Cyanobacterial secondary metabolites with neurotoxic activity can be broadly broken down into two groups based on their structure, (1) the alkaloids, including anatoxin-a and its analog homoanatoxin-a, anatoxin-a(s), and paralytic shellfish poisoning (PSP) toxins (e.g., saxitoxin, gonyautoxin, and their derivatives); and (2) the lipopeptides, including antillatoxins A and B, kalkitoxin, and the jamaicamides. The alkaloid toxins have been isolated from species of *Anabaena*, *Aphanizomenon*, *Microcystis*, *Cylindrospermopsis*, and *Planktothrix* (Sivonen and Jones, 1999) and the lipopeptides, from *Lyngbya majuscula* (Orjala et al., 1995; Berman et al., 1999; Wu et al., 2000; Edwards et al., 2004).

4.3.2. Mode of action/detoxification

In mammals, anatoxin-a and homoanatoxin-a mimic acetylcholine, anatoxin-a(s) blocks acetylcholinesterase activity, the saxitoxins, kalkitoxin, and jamaicamides (A, B, and C) block nerve cell sodium channels, and antillatoxins stimulate sodium channels. Acute toxicosis by any of the alkaloid neurotoxins results in paralysis and eventually death by respiratory distress. A detoxification pathway has not yet been described for the neurotoxins, with the exception of saxitoxin. Saxitoxin toxicity is reduced through the biotransformation of the compound to less potent derivatives (Negri and Jones, 1995) or the inactivation of saxitoxin through conjugation to a protein, saxiphilin (Llewellyn et al., 1997).

4.3.3. Effects/accumulation in aquatic organisms

Alkaloid neurotoxins have been largely investigated in mammals, with mice LD_{50} i.p. values of 375 μg kg^{-1} (anatoxin-a), 250 μg kg^{-1} (homoanatoxin-a), 20 μg kg^{-1} (anatoxin-a(s)), and 10 μg kg^{-1} (saxitoxin, see Kuiper-Goodman et al., 1999, and references therein). Minimal information is available regarding their effects in aquatic organisms; however, anatoxin-a and PSP toxins caused behavioral responses, malformation, and/or mortality in fish and daphnids. Purified saxitoxin concentrations as low as 10 μg L^{-1} delayed hatching of zebrafish (*Danio rerio*), while the highest concentration, 500 μg L^{-1}, caused malformations and mortality. In the same study, anatoxin-a (400 μg L^{-1}) affected zebrafish heart rate, while lower concentrations (40 and 200 μg L^{-1}) had no effect (Oberemm et al., 1999). Whole cells of *Anabaena* sp. (10^7 cells ml^{-1} with 970 μg anatoxin-a g^{-1} dw) were also toxic to fish as all juvenile carp (*Cyprinus carpio*) died after 26–29 h of exposure (Osswald et al., 2007). A lower density of cells (10^5 cells ml^{-1}) did not cause mortality but did produce similar behavioral responses to the higher density treatment, including rapid opercular movement and abnormal swimming. *A. issatschenkoi*, containing PSP toxins, decreased daphnid (*Daphnia magna*) growth and survival (Nogueira et al., 2004). These cyanobacterial alkaloid neurotoxins rapidly accumulate in fish (0.768 μg ANA g^{-1} dw) and mussels (6.2 μg PSP toxins g^{-1} fw), thereby opening the possibility for transfer of these cyanotoxins up the food chain (Negri and Jones, 1995 and Table 3).

The lipopeptides were first described as being acutely ichthyotoxic (antillatoxin A LC_{50} = 0.1 μM, antillatoxin B LC_{50} = 1 μM, kalkitoxin LC_{50} = 0.7 μM, jamaicamides A, B, and C $LC_{100} \geq 5$ ppm) and later identified as neurotoxic (Orjala et al., 1995; Berman et al., 1999; Wu et al., 2000; Nogle et al., 2001; Edwards et al., 2004). Kalkitoxin and jamaicamide C are potent brine shrimp toxins with an LC_{50} of 0.17 μM and LC_{25} = 10 ppm, respectively (Wu et al., 2000; Edwards et al., 2004). Kalkitoxin also inhibits cell division in sea urchin embryos ($IC_{50} \approx 0.025$ μM). It is unknown if these lipopeptides can accumulate in aquatic organisms.

4.4. Dermatoxins/tumor promoters

4.4.1. Structure and source

Lyngbyatoxins and aplysiatoxins are inflammatory agents produced by the marine cyanobacterium *L. majuscula*. Lyngbyatoxins A (LA), B and C are indole alkaloids while aplysiatoxins are phenolic bislactones consisting of aplysiatoxin (AT), debromoaplysiatoxin (DAT), and their less toxic anhydro variants. DAT has also been isolated from other benthic marine cyanobacterium, including *Oscillatoria nigroviridis* and *Schizothrix calcicola*.

4.4.2. Mode of action/detoxification

Lyngbyatoxins and aplysiatoxins act as both dermatoxins, with a mouse LD_{100} of 250 μg kg^{-1} (Ito et al., 2002a), and tumor promoters (Fujiki et al., 1983). Intraperitoneal injection of LA or AT into mice

Table 5
Inhibition of mammalian digestive proteases, trypsin, chymotrypsin and carboxypeptidase A, by secondary metabolites of *Microcystis*, *Anabaena*, *Nostoc*, and *Oscillatoria*/*Planktothrix* sp., reported as IC_{50} values (μg ml^{-1} unless otherwise stated)

Group	Protease Inhibitor	Source(s)	Trypsin	Chymotrypsin	Carboxy. A	Ref.
Group 1	Aeruginopeptins	*M. aeruginosa*, *Microcystis* sp.		**917S-A**, **917S-B**, **917S-C**		7
	Anabaenopeptilides	*A. circinalis*, *A. lemmermannii*		**90-A, 90-B, 202-A, 202-B**		8
	Cyanopeptolins	*M. aeruginosa*	**S** (<0.2), **SS** (<0.2), **A** (<0.2)			1, 2
	Micropeptins	*M. aeruginosa*	**A** (0.071), **B** (0.25), **90** (2.0), **T2** (0.1), **EI992** (3.8), **EI964** (4.2), **SD944** (8.0), **SD999** (4.0),	**T1** (3.0), **88-N** (15 μM), **88-A (0.4)**, **88-Y** (1.3 μM), **T-20** (0.003 μM), **88-C** (5.0), **88-D** (10), **88-E** (5.2), **88-F** (3.4), **SD979** (2.4), **SD1002** (3.2)		1, 3, 4, 9, 11, 12
	Nostopeptins	*N. minutum*		**A** (1.4), **B** (1.6)		6
	Oscillapeptins	*P. rubescens*, *O. agardhii*,	**D** (0.013 μM)	oscillapeptin (2.2)		1, 9
Group 2	Microviridins	*M. viridis*, *M. aeruginosa*	**B** (58), **C** (32), **J** (0.034–0.150)	**B** (2.5), **C** (4.9), **J** (2.8)		1, 10
Group 3	Aeruginosins	*M. aeruginosa*, *Microcystis* sp., *M. viridis*	**298-A** (1.0), **98-A** (0.6), **98-B** (0.6), **98-C** (3.9), **102-A** (0.2), **102-B** (1.1), **EI461** (45.5*)			1, 4
	Microcins	*Microcystis* sp.	**SF608** (0.5)			5
Group 4	Anabaenopeptins	*A. flos-aqua*			**T** (2.0)	3
	Oscillamides	*O. agardhii*		**Y** (10 μM)		1

Groups: (1) Cyclic depsipeptide with Ahp, (2) Tricyclic depsipeptides, (3) Linear peptides with Choi, (4) Cyclic peptides with a ureido linkage. References: (1) Namikoski and Rinehart (1996) (and references therein), (2) Weckesser et al. (1996) (and references therein), (3) Kodani (1999), (4) Ploutno et al. (2002), (5) Banker and Carmeli (1999), (6) Okino et al. (1997), (7) Harada et al. (2001), (8) Harada (2004), (9) Yamaki et al. (2005) (and references therein), (10) Rohrlack et al. (2003), (11) Ishida et al. (1998), (12) Reshef and Carmeli (2001). * indicates IC_{15}.

caused intestinal bleeding, stomach ulcers, and eventually death due to hemorrhagic shock (Ito and Nagai, 2000; Ito et al., 2002a). Topical application with LA, AT, or DAT, on the other hand, resulted in erythema (dermatitis), blisters, and necrosis in mammals. A detoxification pathway has not been identified.

4.4.3. Effects/accumulation in aquatic organisms

LA and DAT have the ability to accumulate in marine grazers, including three species of sea slugs, *Stylocheilus striatus*, *Bursatella leachii*, and *Diniatys dentifer* (Capper et al., 2005 and Table 3). While these toxins can enter the aquatic food chain, the effects of these toxins on fish or aquatic invertebrates have not been tested.

4.5. Irritant toxins

4.5.1. Structure and source

Cyanobacterial lipopolysaccharides (LPS) are generally classified as endotoxins, located within or at the surface of the outer cell layer, and are comprised of a carbohydrate polymer, a core oligosaccharide and an acylated glycolipid (lipid A); the latter segment is linked to toxicity in enteric, heterotrophic Gram-negative bacteria (Stewart et al., 2006, and references therein). Cyanobacteria contain the attributes of both Gram-positive and Gram-negative bacteria (Stewart et al., 2006, and references therein) and toxic LPS have been isolated from numerous genera of cyanobacteria including *Microcystis*, *Anabaena*, *Spirulina*, and *Oscillatoria*. The LD_{50} values of cyanobacterial LPS in mice ranged from 45 to 190 mg kg^{-1} (Keleti and Sykora, 1982; Raziuddin et al., 1983; Tornabene et al., 1985).

4.5.2. Mode of action/detoxification

Little is known about the intoxication or detoxification pathway of cyanobacterial LPS, and instead, assumptions are based on the vast knowledge of Gram-negative bacterial LPS. The latter indirectly cause fever, septic shock syndrome, inflammation, toxicant-induced liver damage, and reduce pools of detoxification enzymes (i.e., cytochrome *P450* and glutathione-*S*-transferase) in mammals. Cyanobacterial LPS have been associated with numerous human illnesses, ranging from skin irritation to gastrointestinal tract and respiratory distress; however, these associations are anecdotal and require additional research before the health effects of cyanobacterial LPS in humans are considered definitive (Stewart et al., 2006).

4.5.3. Effects/accumulation in aquatic organisms

Raziuddin et al. (1983) demonstrated that cyanobacterial LPS are less toxic than Gram-negative bacterial LPS; however, LPS from *Microcystis* and *Gleotrichia* were more potent inhibitors of glutathione-*S*-transferase activity in fish embryos than were LPS from Gram-negative bacterial sources, *Salmonella tsyphimurium* and *E. coli* (Best et al., 2002). When co-administered with microcystin-LR, *Microcystis* LPS decreased hepatotoxin potency in *A. salina*, *D. magna*, and *D. galeata* (Lindsay et al., 2006). Additional research is necessary to investigate the effects of cyanobacterial LPS on aquaculture species, as these irritant toxins are a common component of cyanobacterial cell walls. To date, there are no reports of LPS accumulation in aquatic organisms.

4.6. Protease inhibitors

4.6.1. Structure and source

Numerous secondary metabolites have been isolated from cyanobacteria which inhibit the enzymatic digestion of proteins (i.e., protease inhibitors). Protease inhibitors can be produced by both toxic strains (e.g., those that produce hepatotoxins or neurotoxins) and non-toxic strains of *Microcystis*, *Anabaena*, *Planktothrix*/*Oscillatoria*, and *Nostoc*. Namikoshi and Rinehart (1996) classified serine protease inhibitors into four groups based on their structural characteristics (Table 5). Members of Group 1 are cyclic depsipeptides with the unique amino acid 3-amino-6-hydroxy-2-piperidone (Ahp), and include variants of micropeptins, cyanopeptolins, oscillapeptins, nostopeptins, aeruginopeptins, and anabaenopeptilides. Microviridins, tricyclic depsipeptides, make up Group 2. Group 3 consists of linear peptides with a unique amino acid unit 2-carboxy-6-hydroxy-octahydroindole (Choi), namely aeruginosins and microcins. Group 4 consists of anabaenopeptins and oscillamides, cyclic peptides with an ureido linkage.

4.6.2. Mode of action/detoxification

Inhibition occurs through the binding and subsequent inactivation of a serine protease enzyme: trypsin, chymotrypsin, and/or

carboxypeptidase A (Table 5). When crystallized with bovine trypsin, A90720A, a variant of Group 1 from a terrestrial bacteria, interacted with the protease in a series of hydrogen bonds, hydrophobic interactions and steric interference, with the Ahp unit being essential for bioactivity (Bonjouklian et al., 1996). Specificity for trypsin or chymotrypsin required corresponding hydrophilic amino acids (Arg, Lys) or hydrophobic amino acids (Ile, Leu, Val, Phe) to be in the variable amino acid position in the Group 1 inhibitors (Yamaki et al., 2005). The unique amino acid (Choi) of the Group 3 inhibitors interacts with the catalytic site of the target protease (Sandler et al., 1998). Variants of Group 2 and Group 3 also inhibit trypsin (microviridin), chymotrypsin (microviridin and oscillamide Y), and carboxypeptidase A (anabaenopeptin). A detoxification pathway has not been reported for these cyanobacterial protease inhibitors.

4.6.3. Effects/accumulation in aquatic organisms

While the effects of protease inhibitors have been well studied in mammals (Table 5), fewer studies have focused on daphnids, and none directly on aquaculture species. The research conducted on daphnids, however, is valuable as (1) daphnids can be important prey (food source) in extensive aquaculture systems, and (2) daphnids may be an appropriate model for aquaculture species as digestive enzymes are generally well conserved between animals. Daphnid exposure to protease inhibitors may be common during cyanobacterial blooms, as 70% of the 89 *Planktothrix* strains tested contained inhibitors of daphnid trypsin (Rohrlack et al., 2005) and multiple strains of *Microcystis* sp. inhibited proteases of daphnids or another cladoceran, *Moina macrocopa* (Agrawal et al., 2001, 2005a,b; Rohrlack et al., 2003).

Protease inhibition causes an incomplete digestion of proteins, resulting in negative effects on nutrition, growth, and other metabolic processes that depend on protease activity, including enzyme activation/deactivation (e.g., through partial digestion) or protein recycling. Exposure to microcin SF608 (Group 3) reduced the activity of a detoxification enzyme, soluble glutathione-*S*-transferase (sGST), in *D. magna* (Wiegand et al., 2002). Wiegand et al. proposed that microcin inhibited serine proteases responsible for the partial digestion sGST, causing the affected enzymes to remain in an inactive form. Inhibition of this important detoxification pathway would have major implications for the metabolism of other xenobiotics, such as microcystins which are detoxified via this pathway in daphnids, fish and mammals (Section 4.1). Microviridin J (Group 2) caused lethal molting failure in *Daphnia pulicaria* (Rohrlack et al., 2004). Microviridin J inhibited proteases responsible for (1) the digestion of previous integuments, disrupting new molting processes and/or (2) the digestion of nutritional protein, leading to insufficient amino acids for molt regeneration. Other crustaceans (e.g., crab, shrimp, and crayfish) which also undergo molts should be investigated for molting failure. Additional research is necessary to understand the severity of nutritional deficiency caused by protease inhibitors *in situ* and explore the possibilities for indirect effects on growth, reproduction, and survival.

Elastase, plasmin, tyrosinase, papain and thrombin are other serine or cysteine proteases inhibited by secondary metabolites isolated from cyanobacteria. These protease inhibitors have numerous pharmaceutical and industrial (e.g., fungicide) applications (Tan, 2007); however, the possible relationship between their production and aquaculture species is unclear. As a result, these compounds were outside the scope of this review (refer to reviews by Namikoshi and Rinehart, 1996; Weckesser et al., 1996; Rao et al., 2002).

4.7. Grazer deterrents

4.7.1. Structure and source

Numerous secondary metabolites have been isolated from the benthic marine cyanobacterium *L. majuscula* that act as broad grazer deterrents, including (1) the cyclic depsipeptide, pitipeptolide (Luesch et al., 2001), (2) the lipopeptides, ypaoamide (Nagle et al., 1996), microcolin B (Koehn et al., 1992), malyngamides A and B (Todd and Gerwick, 1995), and majusculamides A and B (Marner et al., 1977), and (3) a lipid, malyngolide (Cardllina et al., 1979).

4.7.2. Mode of action/detoxification

The modes of action or inactivation of these grazer deterrent compounds have not been reported.

4.7.3. Effects on aquatic organisms

L. majuscula is not a preferred food for many grazers, allowing dense mats of the benthic cyanobacterium to bloom and persist in areas containing high densities of herbivores (Nagle and Paul, 1998). As a result, secondary metabolites of *L. majuscula* were investigated as a chemical defense against grazing. Herbivores were simultaneously offered (1) a desirable food that was artificially spiked with a compound isolated from *L. majuscula* and (2) an identical food that did not contain the metabolite. A compound was considered a deterrent if grazers consumed more of the food which did not contain the metabolite relative to that which was spiked (i.e., selectively avoiding the deterrent). A natural concentration of pitipeptolide A deterred grazing by four invertebrates, the sea urchin *Echinometra mathaei*, the crab *Menaethius monoceros*, and two amphipods *Parhyale hawaiensis* and *Cymadusa imbroglio* (Cruz-Rivera and Paul, 2007). Feeding preference by the sea hare *S. striatus*, however, was not affected by pitipeptolide A. Ypaoamide also was a broad deterrent, affecting a species of sea hare, *S. longicauda*, three species of herbivorous fish, *Siganus argeneus*, *S. spinus*, and *Scarus schlegei*, and the sea urchin, *E. mahaei* (Nagle et al., 1996; Nagle and Paul, 1998). Malyngamides A and B, malyngolide, majusculamides A and B, and microcolin B all deterred feeding of the sea hare, *S. longicauda* (Nagle et al., 1998), and with the exception of the latter, all have been shown to reduce feeding in reef fish (Paul and Pennings, 1991; Pennings et al., 1996; Thacker et al., 1997). At low concentrations, however, many of the compounds stimulated feeding by the sea hare, a known grazer of *L. majuscula* (Nagle et al., 1998).

Blooms of *L. majuscula* can form in aquaculture systems as grazers selectively avoid this cyanobacterium. As suitable food alternatives become scarce, cultured organisms are more likely to consume the benthic cyanobacterium, having possible implications for growth (e.g., cyanobacteria have poor food quality, Ravet and Brett, 2006), product quality (e.g., *Lyngbya* sp. can produce odorous metabolites, Table 4), and/or health (i.e., *Lyngbya* sp. can produce other bioactive metabolites, Sections 4.2, 4.3, 4.4). Malyngamides and majusculamides have been isolated from the sea hare, *S. longicauda*, showing that these deterrents can be accumulated in the food chain (Paul and Pennings, 1991; Pennings and Paul, 1993; Pennings et al., 1996); however, possible effects on higher trophic levels have not been investigated. The production of chemical defense compounds extends beyond *L. majuscula*; an extracellular compound of *A. flos-aquae* decreased filtering rates (i.e., feeding rates) of *Daphnia pulex* (Ostrofsky et al., 1983) and zooplankton significantly decreased filtering rates when exposed to toxic strains, as compared to non-toxic strains, of *Microcystis* (DeMott et al., 1991) and *Nodularia* (Engström et al., 2000). The latter two studies, however, did not determine whether the reduction was due to strain differences in food quality or in the production of a chemical deterrent and/or toxin.

4.8. Brine shrimp/molluskal toxins

Secondary metabolites have been isolated from *L. majuscula* that are potent toxins against the mollusk (snail) *Biomphalaria glabrata* (barbamide) or brine shrimp, *A. salina* (taveuniamides and curacins). These toxins are diverse in structure, including the chlorinated lipids, taveuniamides (LC_{50} = 1.7–4.2 μg ml^{-1}, Williamson et al., 2004), the chlorinated lipopeptide, barbamide (LC_{100} = 10 μg ml^{-1}, Orjala and Gerwick, 1996), and the lipids curacin A and D (LC_{50} = 0.003–0.03 μg ml^{-1}, Gerwick et al., 1994; Marquez et al., 1998). The mode of toxicity or detoxification has not been identified for these compounds. These toxins

have implications for aquaculture if the snail or brine shrimp are representative of cultured crustaceans and mollusks, such as crayfish, shrimp, crabs, mussels or clams. Other metabolites, such as antillatoxin, kalkitoxin, and jamaicamides, were originally isolated based upon their activity against brine shrimp and/or fish and then later classified as neurotoxic with additional research (Section 4.3).

5. Management

All the cyanobacterial secondary metabolites discussed in this review have the potential to decrease productivity in aquaculture systems and therefore require management (Fig. 1). Mortality of target organisms equates to loss of product and revenue for aquaculture (hepatotoxins, neurotoxins, cytotoxins, brine shrimp/molluskal toxins). Growth rates of target species may decrease as a response to decreased nutritional intake (protease inhibitors, grazer deterrents) or the re-allocation of energy to detoxification and/or repair processes (hepatotoxins, cytotoxins, neurotoxins, dermatoxins, irritant toxins). A decreased growth rate, in turn, means more resources, such as labor, food, or pond space, are required for the product to reach the appropriate size/maturity before an optimal value can be returned. A compromised immunity, whether caused directly (microcin) or indirectly (all harmful metabolites reviewed), could also decrease productivity by increasing susceptibility to other adverse biological, chemical, or physical agents in the aquatic environment. For example, susceptibility to disease increases under stress conditions (Schreck et al., 2001). Intensive aquaculture systems are most susceptible to the risks associated with these bioactive metabolites as dense stocking and hypoxia are well established stresses. Products with an unfavorable odor (MIB, geosmin), malformations (hepatotoxins, saxitoxin), or a disfigured appearance, for example with tumors or lesions (microcystins), can have a lowered economic value due to public perception and/or the cost of subsequent remediation of the product. The removal of toxin-laden organs or tissue from target organisms also will inflate costs (hepatotoxins, cytotoxins, neurotoxins, dermatoxins). Hepatotoxins, especially, have been found to persist in the flesh of aquatic animals for weeks to months after their removal to a pristine environment (Eriksson et al., 1989; Yokoyama and Park, 2003; Dionisio Pires et al., 2004; Xie et al., 2004; Soares et al., 2004). The half-life for microcystins in molluskal tissue ranges from 3.1–8.4 d, with the depuration rate decreasing at lower temperatures (Yokoyama and Park, 2003; Ozawa et al., 2003). More research to address the occurrence and severity of these processes in both aquaculture and natural systems is needed.

The control of cyanobacteria has been an elusive goal, particularly in intensive aquaculture. Most of these operations are located in tropical–temperate locations, with conditions suitable for algal proliferation. Systems receive large nutrient inputs from unassimilated feed and optimizing feeding rates would reduce nutrients for algal growth. Two methods have been employed to control noxious algal blooms or remedy their effects — the reduction of undesirable algae via physical/chemical methods, and post-harvest treatment techniques. Physical methods have included moving fish to clean water to allow purging from tissues (Johnsen and Lloyd, 1992), use of planktivorous fish stocked with the more desirable species, flushing of enclosures to prevent vertical thermal stratification and reduce algal concentrations (Mitrovic et al., 2006), and separation of fish from algae in partitioned aquaculture systems (Brune et al., 2004; Mueller et al., 2004). It is rare for freshwater aquaculture operations to have sufficient free space to move non-saleable fish to pristine pond water. The resources needed to seine and move these animals and the stress or death inflicted on the animals exceed potential returns. In marine and brackish aquaculture systems, however, this method is readily practiced with success.

Chemical methods, such as low-level copper treatments, can be used to remove undesirable cyanobacterial species and allow sufficient time for cultured species to depurate odorous (off-flavor) or bioactive metabolites. Copper is currently the only approved product for use in all aquaculture. Low-level copper doses (weekly applications of 1 ppm copper) reduce the incidence of off-flavor products (Schrader et al., 2005). Copper resistance has been exhibited in algae that survived low-level treatments (Vymazal 1984; García-Villada et al., 2004), suggesting this treatment may not be sustainable in the long term. Many countries have limited copper use in potable waters, and many alternative treatments are being considered to reduce copper usage. Diuron, the active ingredient in urea-based herbicides, has been approved for control of off-flavor producing algae in the United States; diuron inhibits photosystem II. Filamentous cyanobacteria (including the most common off-flavor producing cyanobacteria in Mississippi) were controlled using repeated diuron treatments, however treatment enhanced growth of coccoid cyanobacteria (Zimba et al., 2002). The development of new herbicides is possible (Schrader et al., 2000; Zimba et al., 2002; Schrader and Tucker, 2003), but requires governmental approval of the treatment which can slow implementation. The shallow depths of most aquaculture ponds preclude the use of pressure to control gas-vacuolated cyanobacterial species (Porat et al., 1999), however, ultrasound technologies have great potential (Tang et al., 2004; Zimba, pers. obs.). Additionally, technologies such as titanium dioxide may be applicable to the management of algal populations (Kim and Lee, 2005). A practical remedy is to deliver feed upwind of dense blooms, preventing fish exposure to windrowed algae in the short term.

Post-harvest chemical treatment of fish to remove off-flavor metabolites using various solvents has been attempted by several groups with limited success (Grimm, pers. comm.). Flavored coatings, such as lemon/pepper, can mask off-flavors (Bett et al., 2000; Joan King, pers. comm.), however their routine usage in processing facilities has not occurred. Practical limitations of tracking different lots of fish to assure prophylactic treatments are difficult within most processing facilities.

It is clear that aquacultured food supplies are critical to meet the protein demands of an expanding world population. Growth of the aquaculture industry will continue to require sound environmental stewardship, development of genetically superior strains able to survive and grow quickly under suboptimal pond conditions, and development of technologies for controlling nuisance algal species.

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